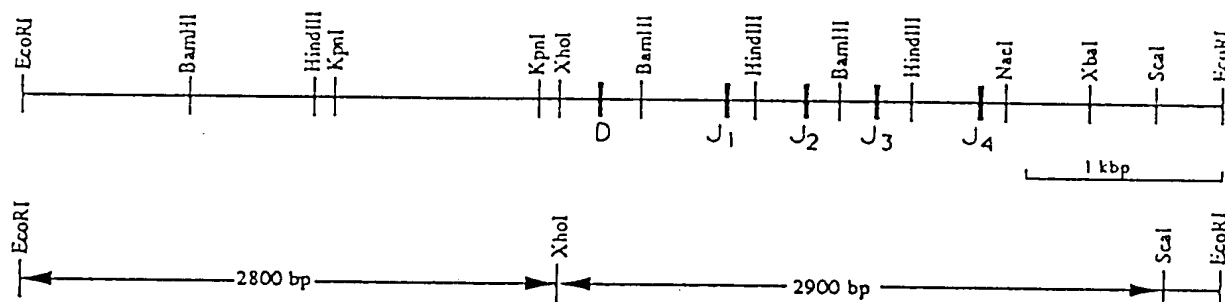


Mouse Heavy Chain J Genes Inactivation Vector

(A) Targeted mouse heavy chain J genes



(B) Inactivation vector mDAJ.Neo



Figure 1

(A) Targeted mouse heavy chain I genes

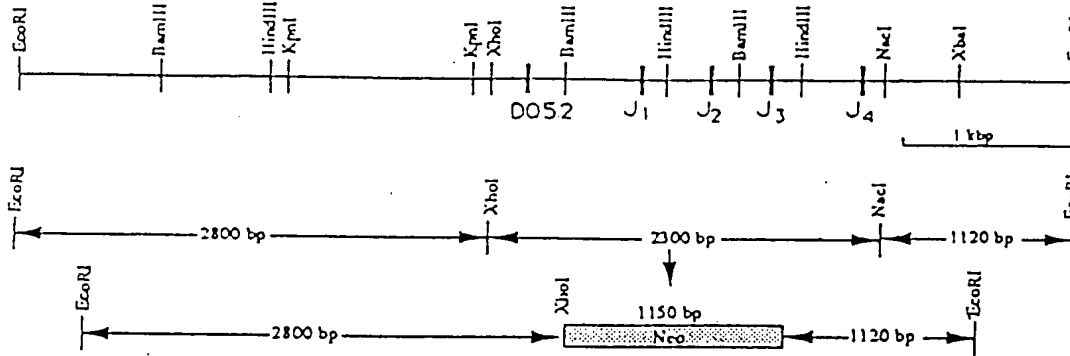
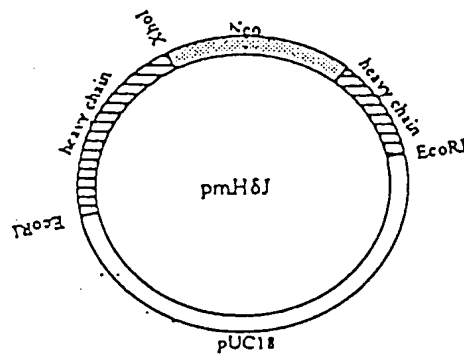
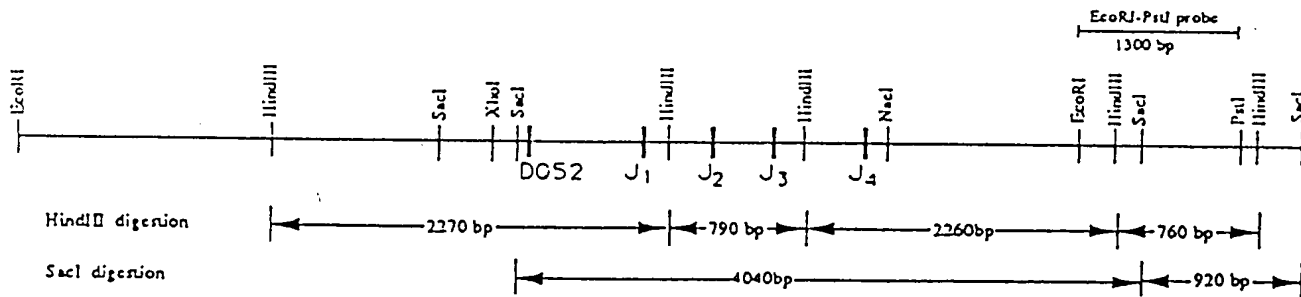
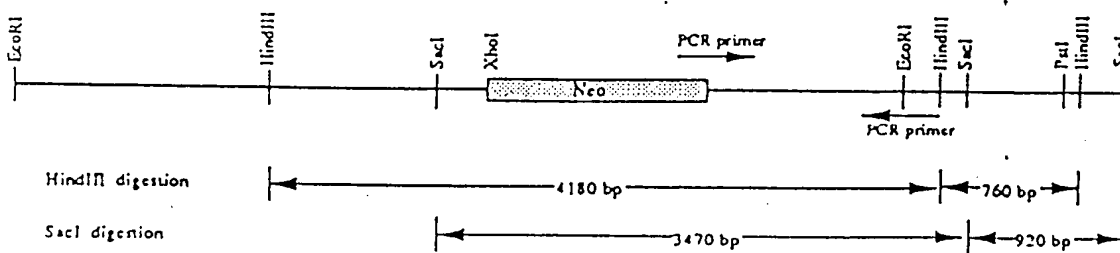
(B) Inactivation vector pmH δ J(C) Southern analysis of pmH δ J-targeted ES coloniesWild type ES cell genomeTargeted ES cell genome

Figure 2

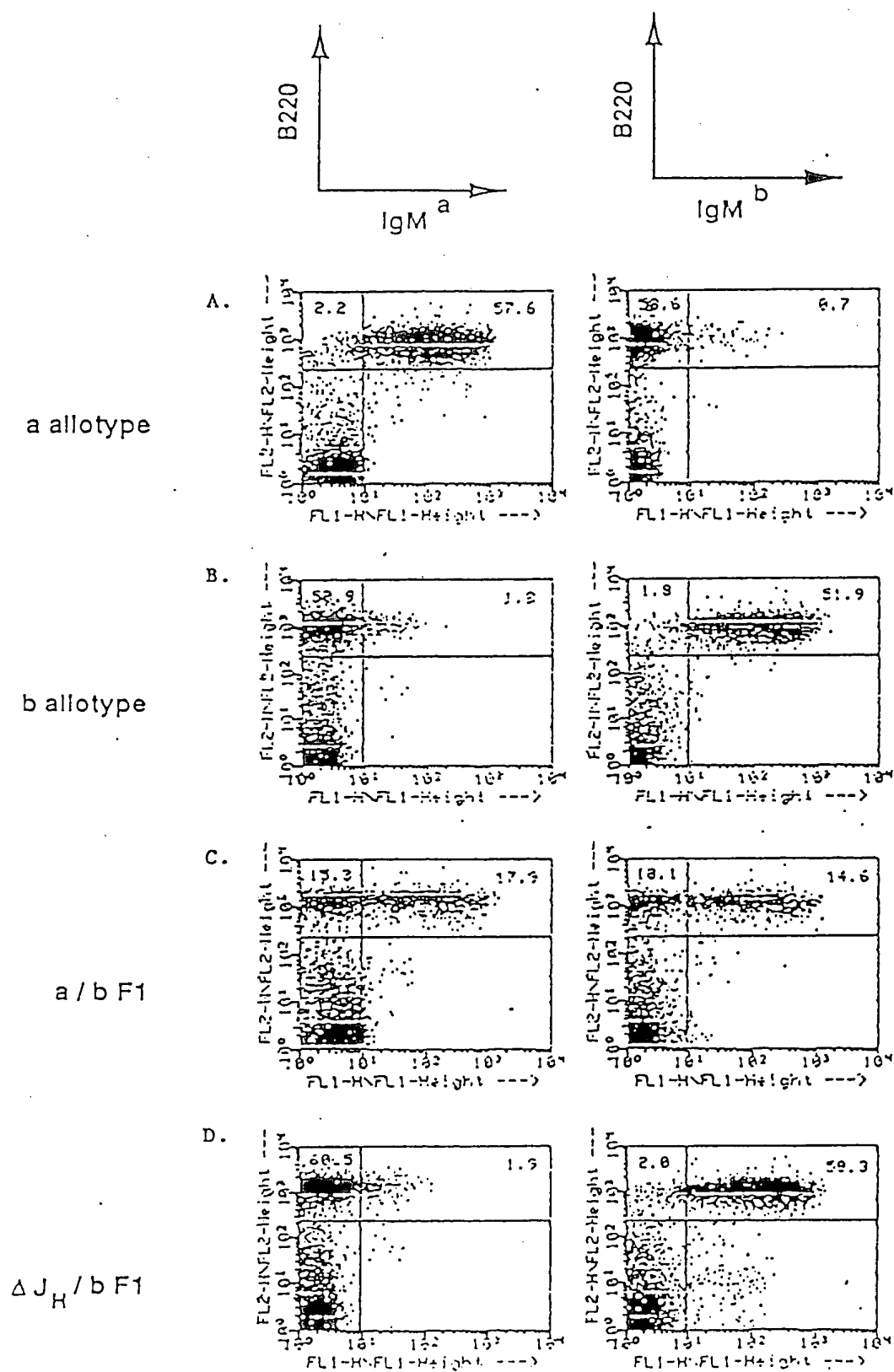
J_H deletion blocks cell surface IgM expression

Figure 3

Staining of peripheral blood lymphocytes with fluorescent anti-a allotype (A, D), anti-b allotype (B, E) or anti-B220 (C, F). (A, B, C) JH-deletion homozygous mutant mouse 244-3-2/F2-7, (D) A allotype control mouse, (E) B allotype control mouse. The number in each panel indicates the percentage of cells stained with the specific antibody.

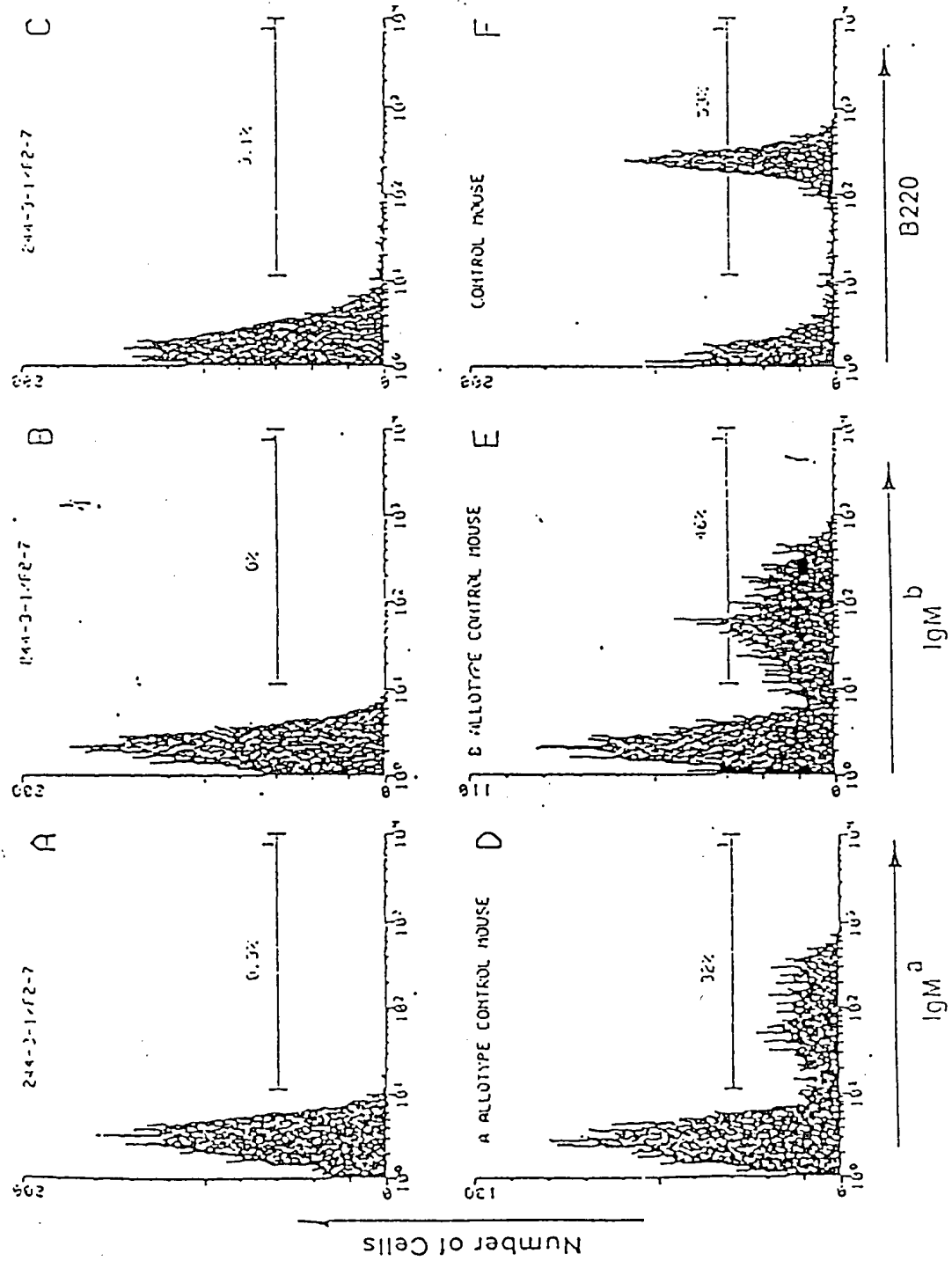


Figure 4

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INACTIVATION OF KAPPA CONSTANT REGION

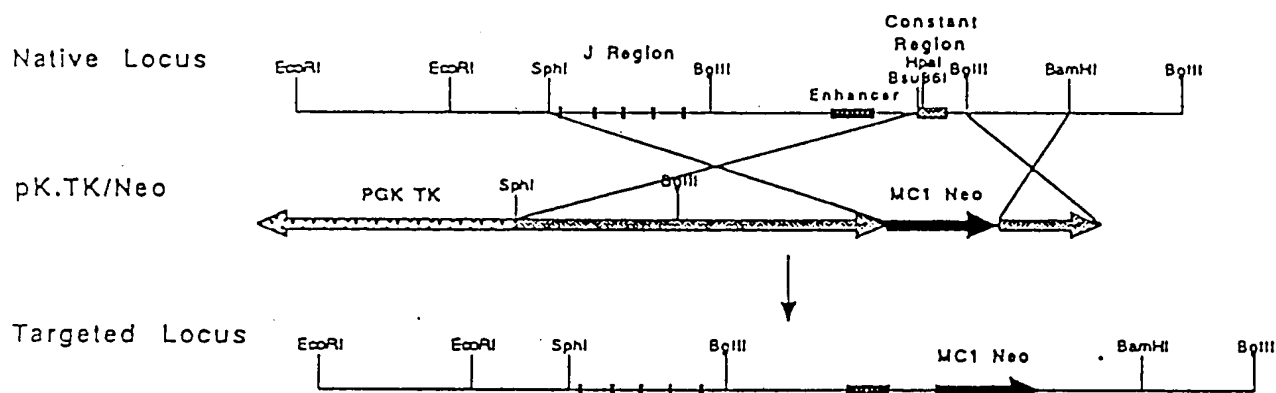


Figure 5

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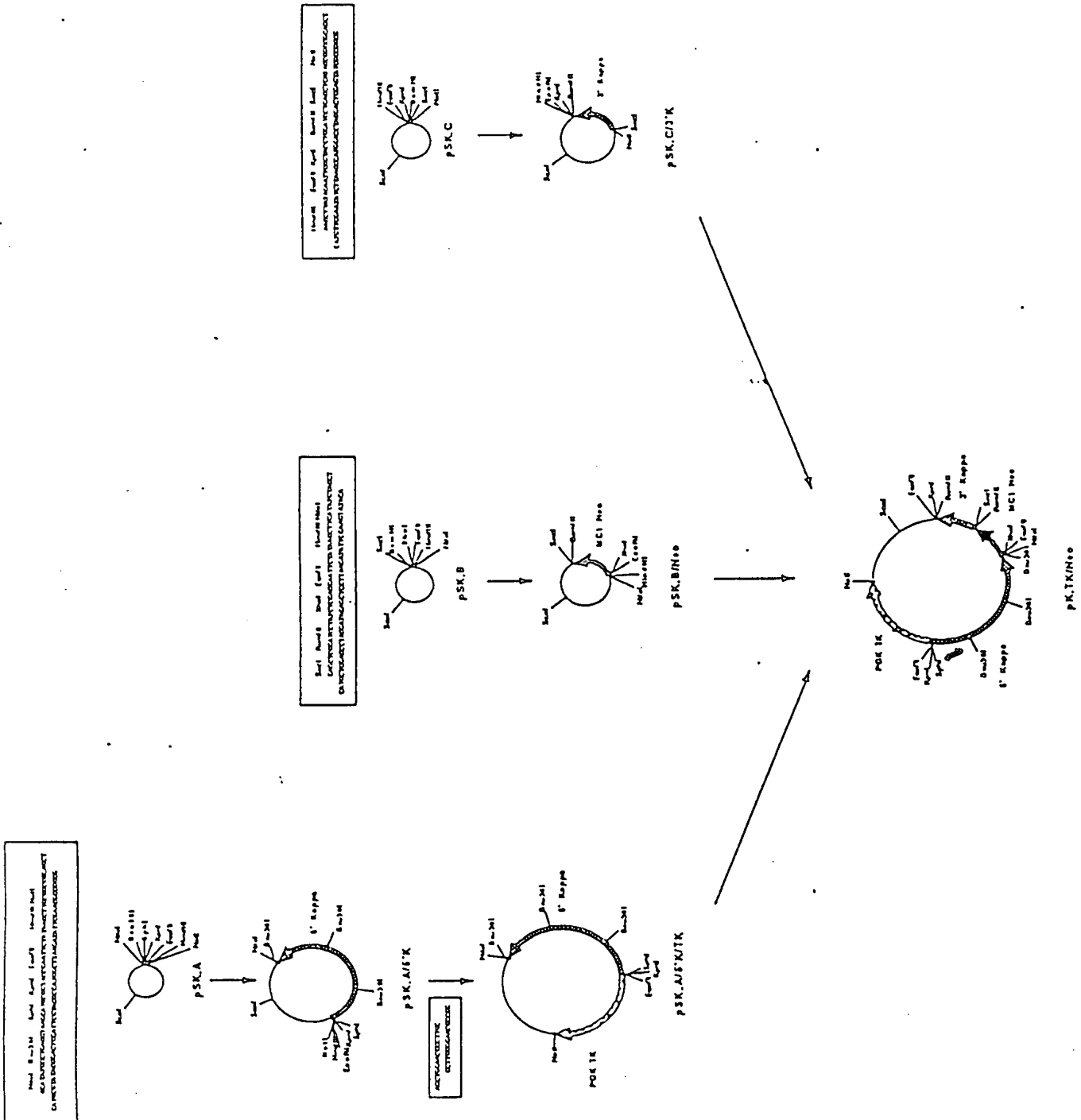
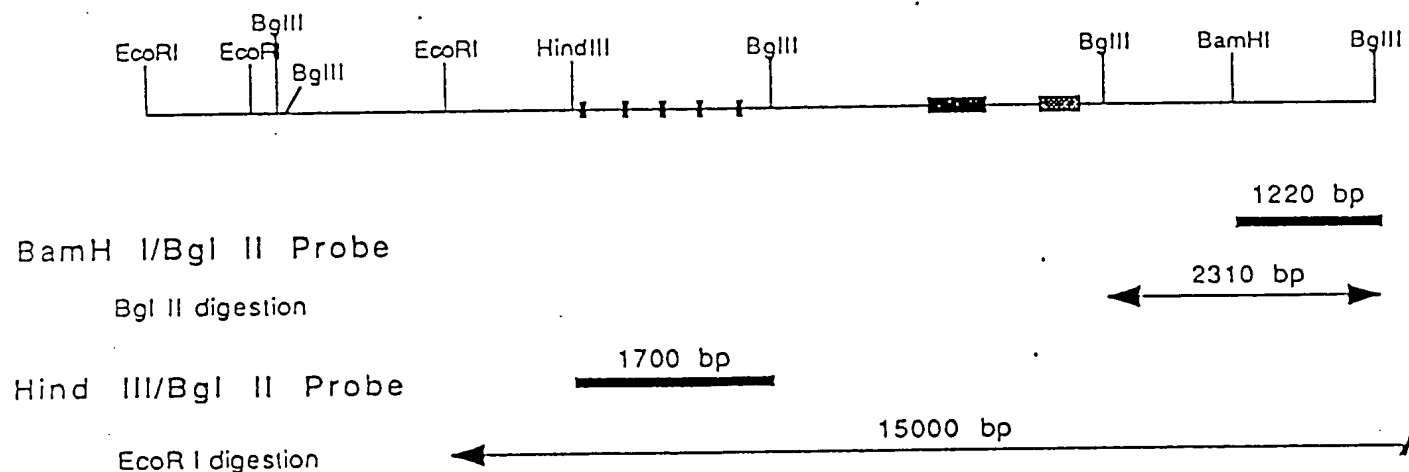


Figure 6

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SOUTHERN ANALYSIS OF LIGHT CHAIN C κ -TARGETED E14-1 CELLS

NATIVE ES CELL LOCUS



TARGETED ES CELL LOCUS

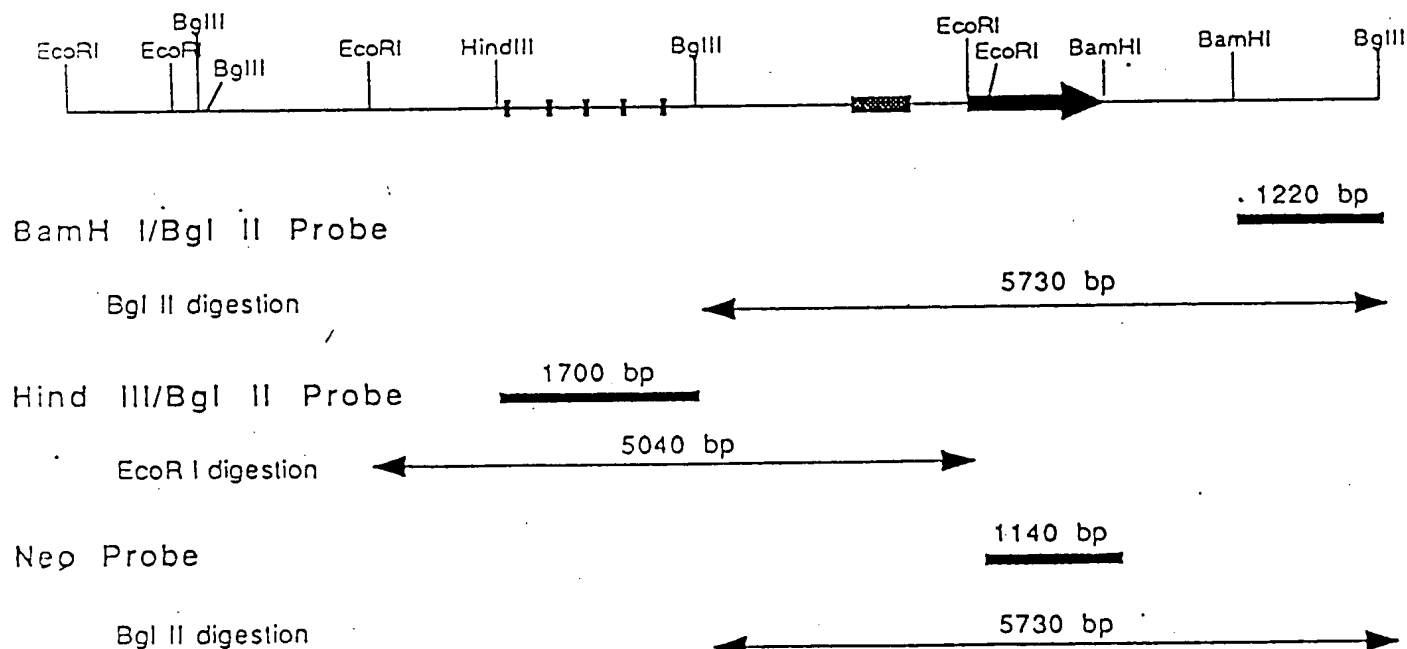
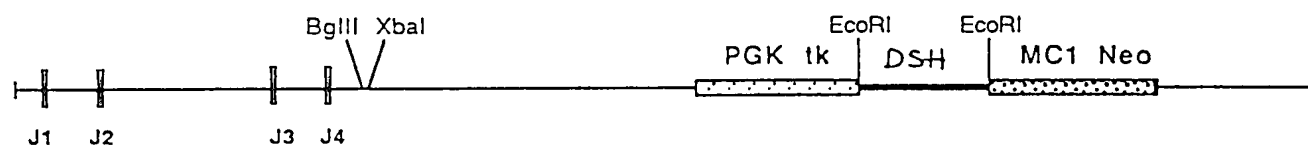


Figure 7

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KAPPA J/CONSTANT REGION INACTIVATION

J REGION KNOCKOUT VECTOR



TARGETING SCHEME

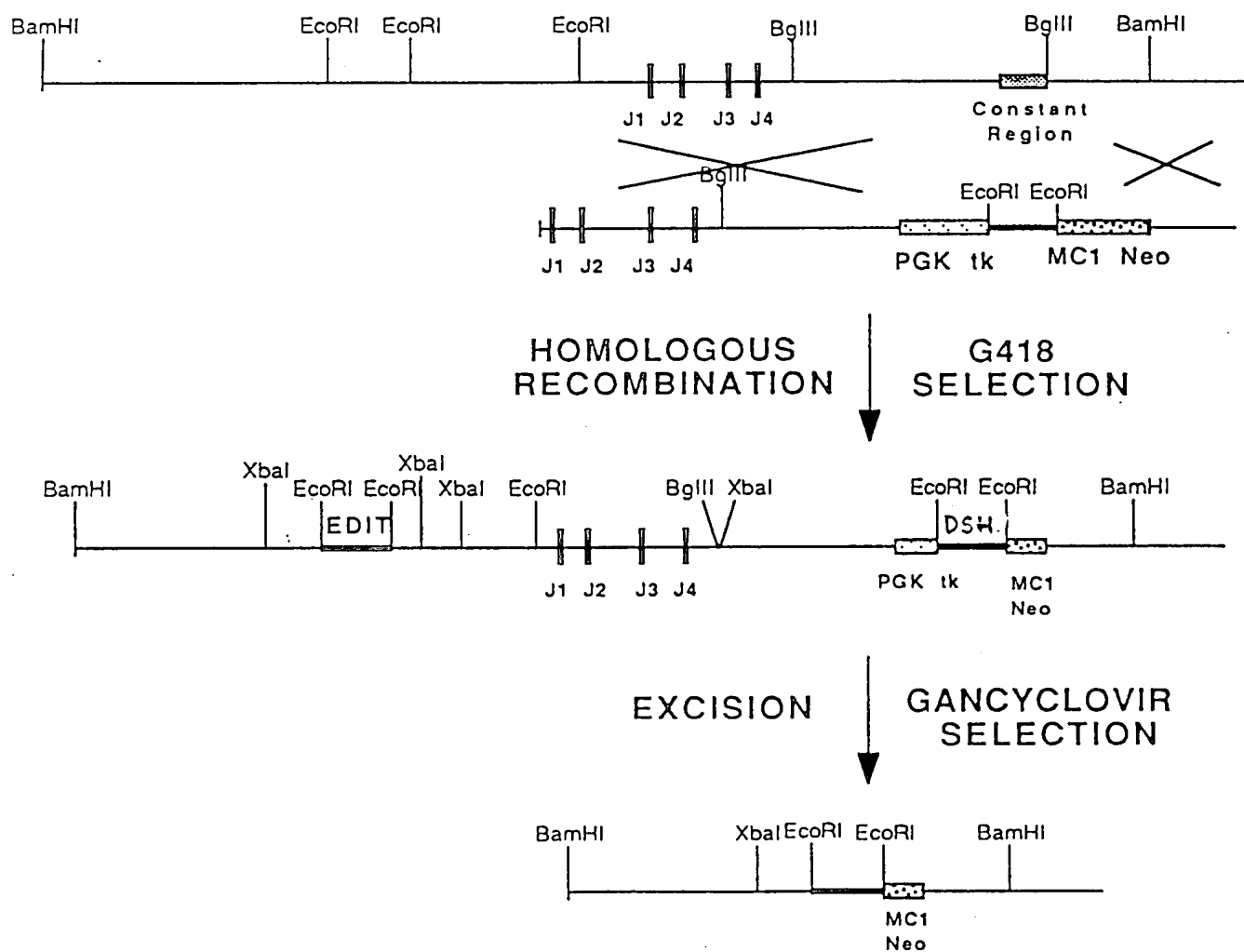


Figure 8

Figure 9

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KAPPA J/CONSTANT REGION DELETION VECTORS

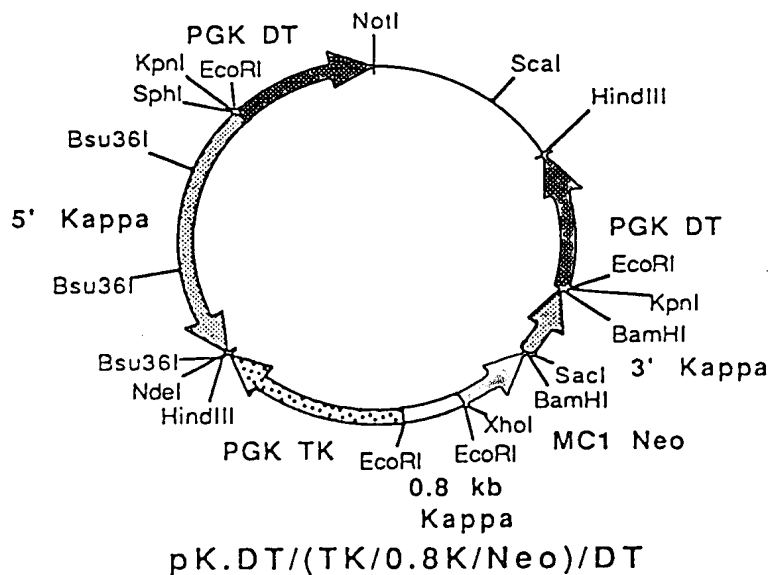
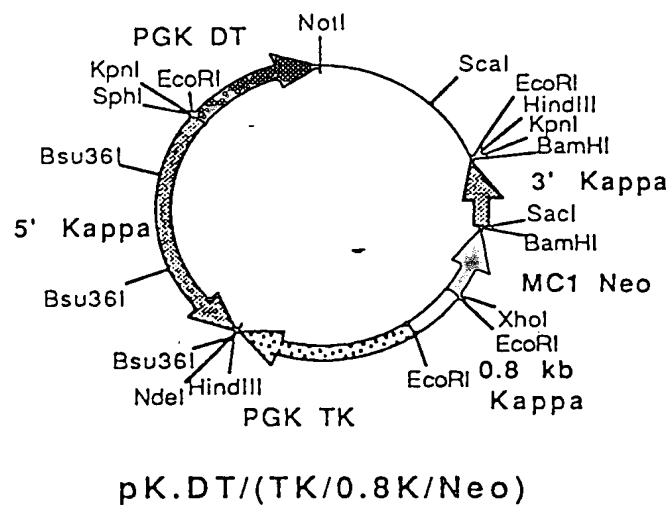
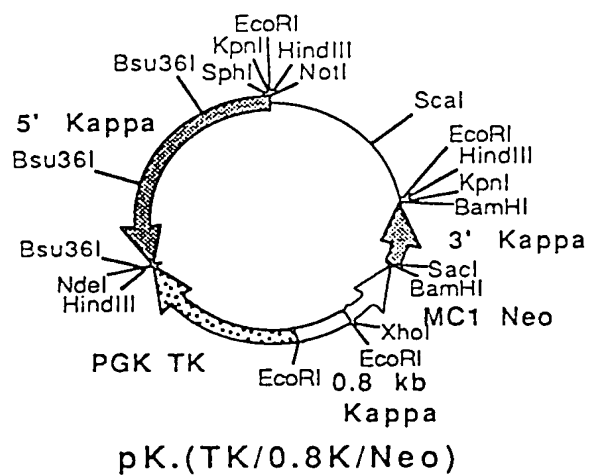
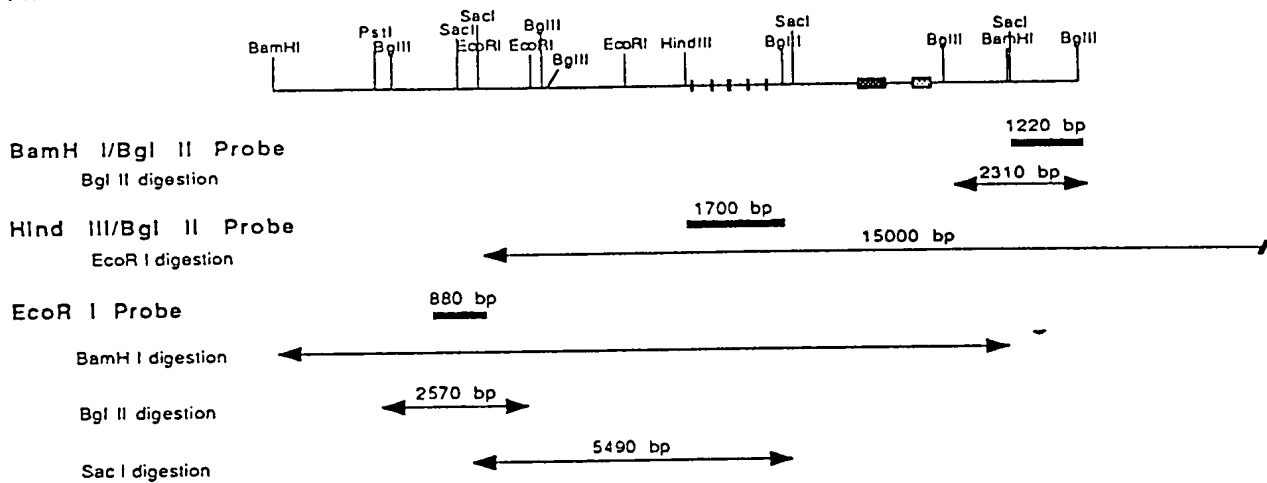


Figure 10

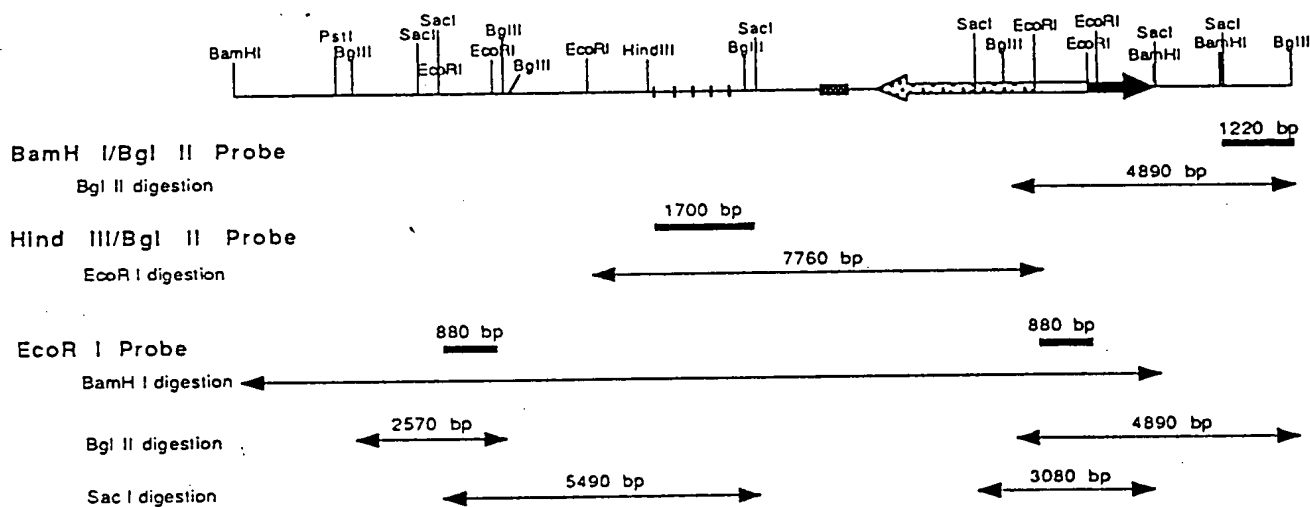
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SOUTHERN ANALYSIS OF LIGHT CHAIN J κ /C κ -DELETED E14-1 CELLS

NATIVE ES CELL LOCUS



C κ -TARGETED ES CELL LOCUS



J κ C κ -DELETED ES CELL LOCUS

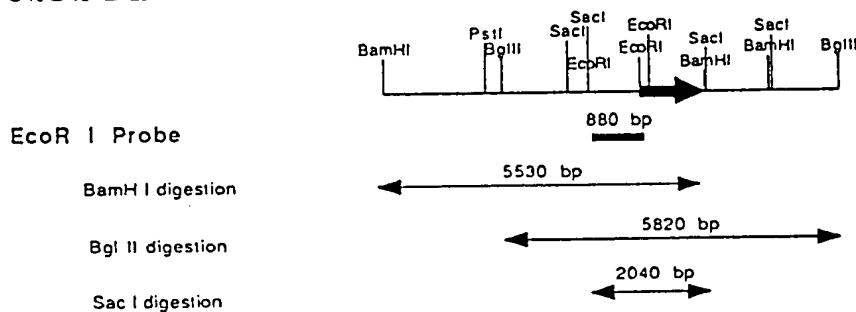


Figure 11

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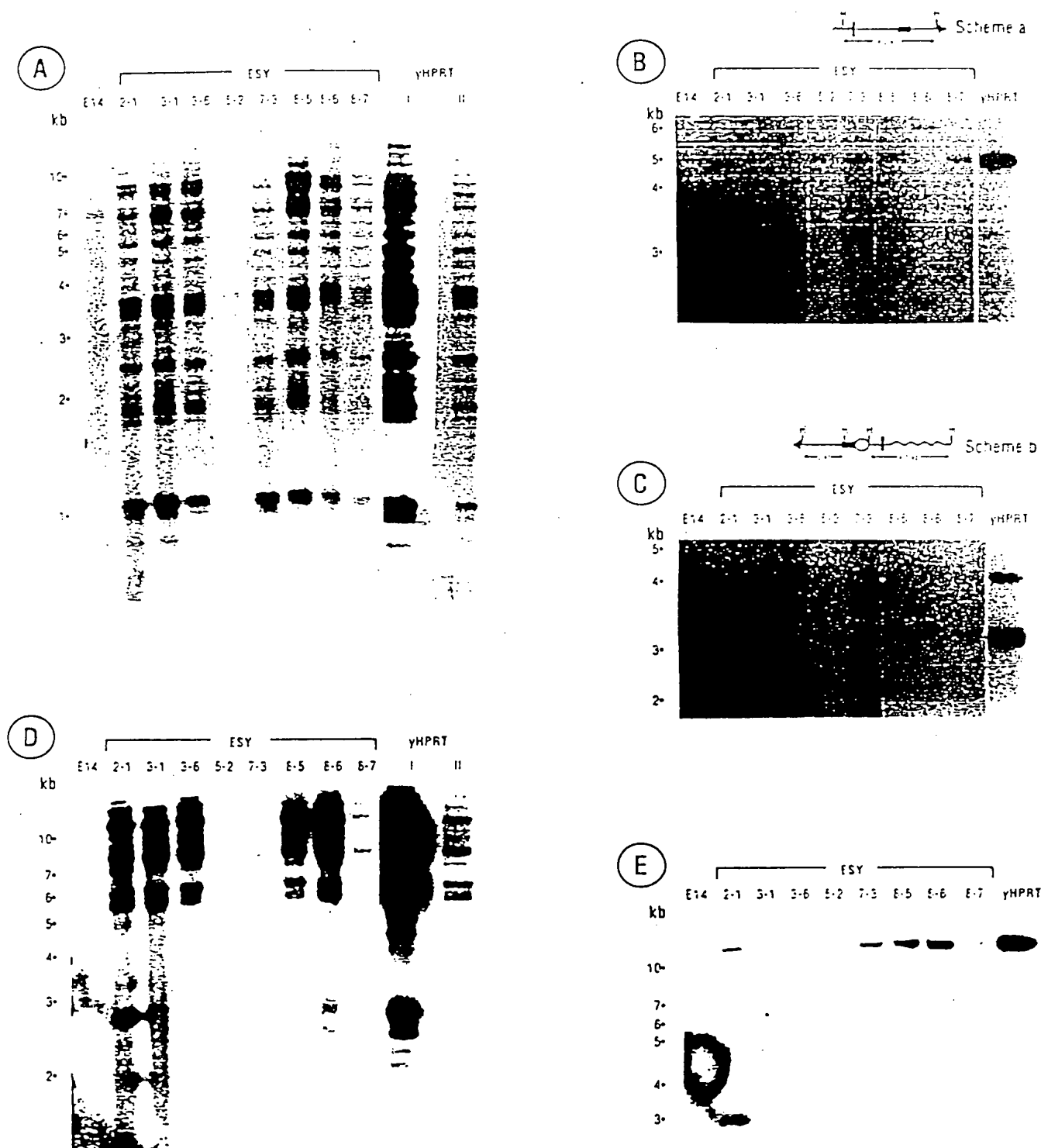


Figure 12

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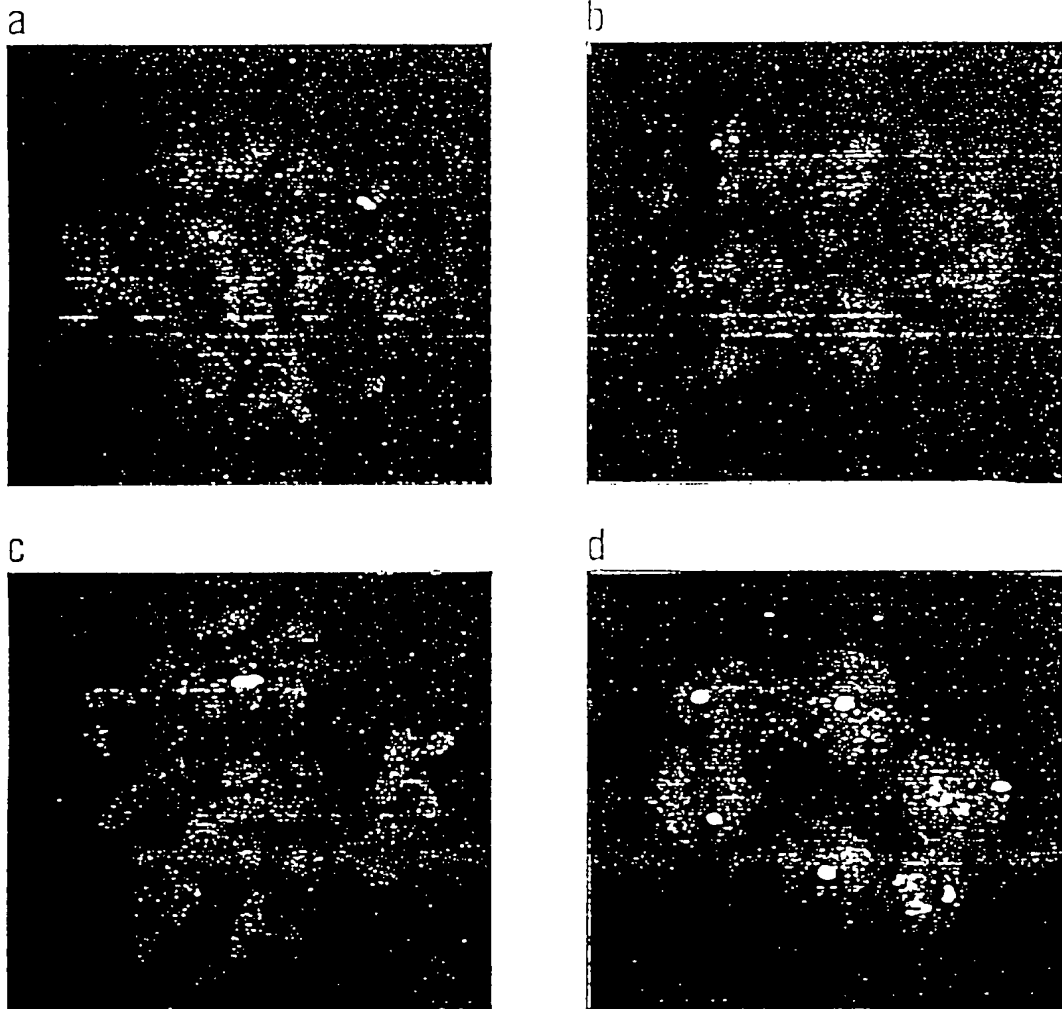


Figure 13

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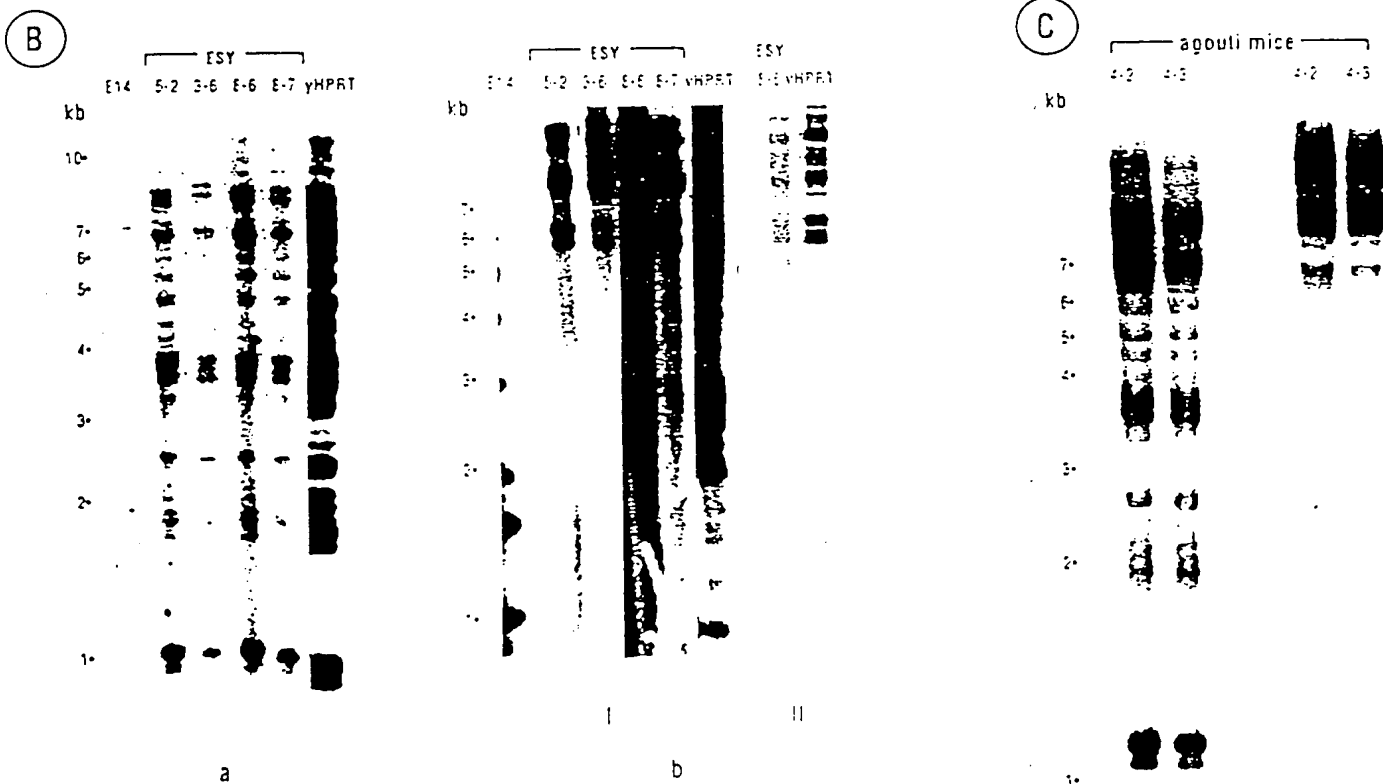
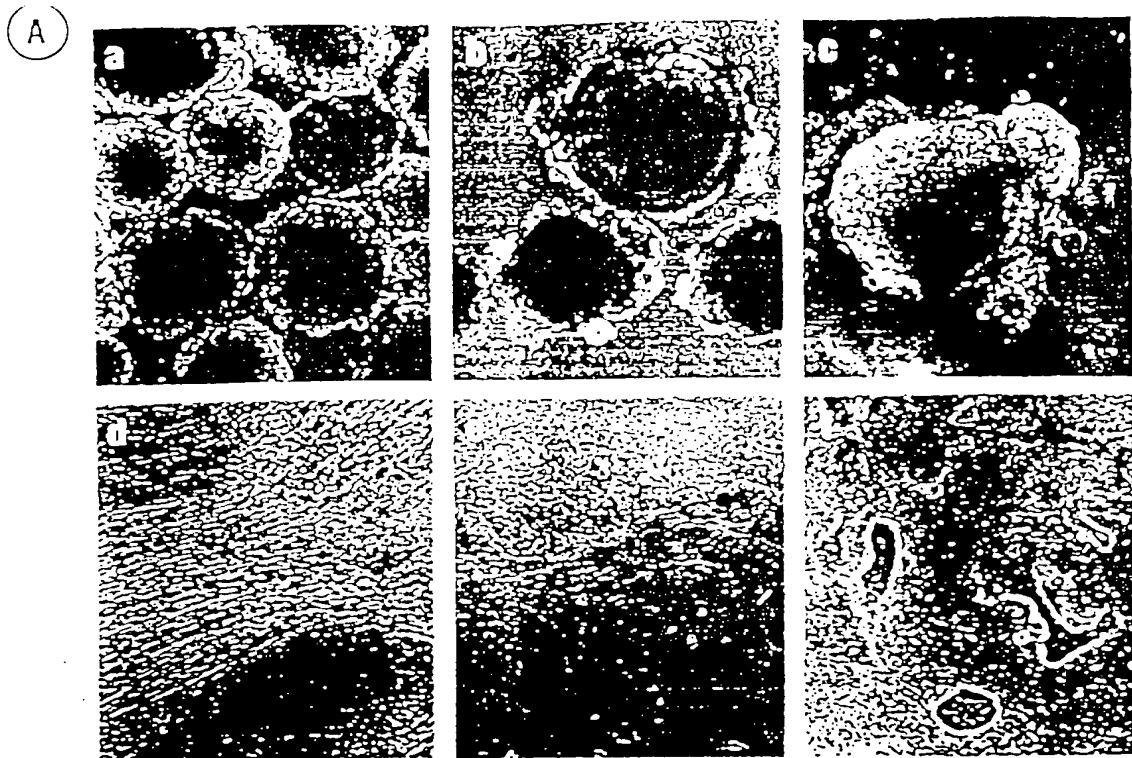


Figure 14

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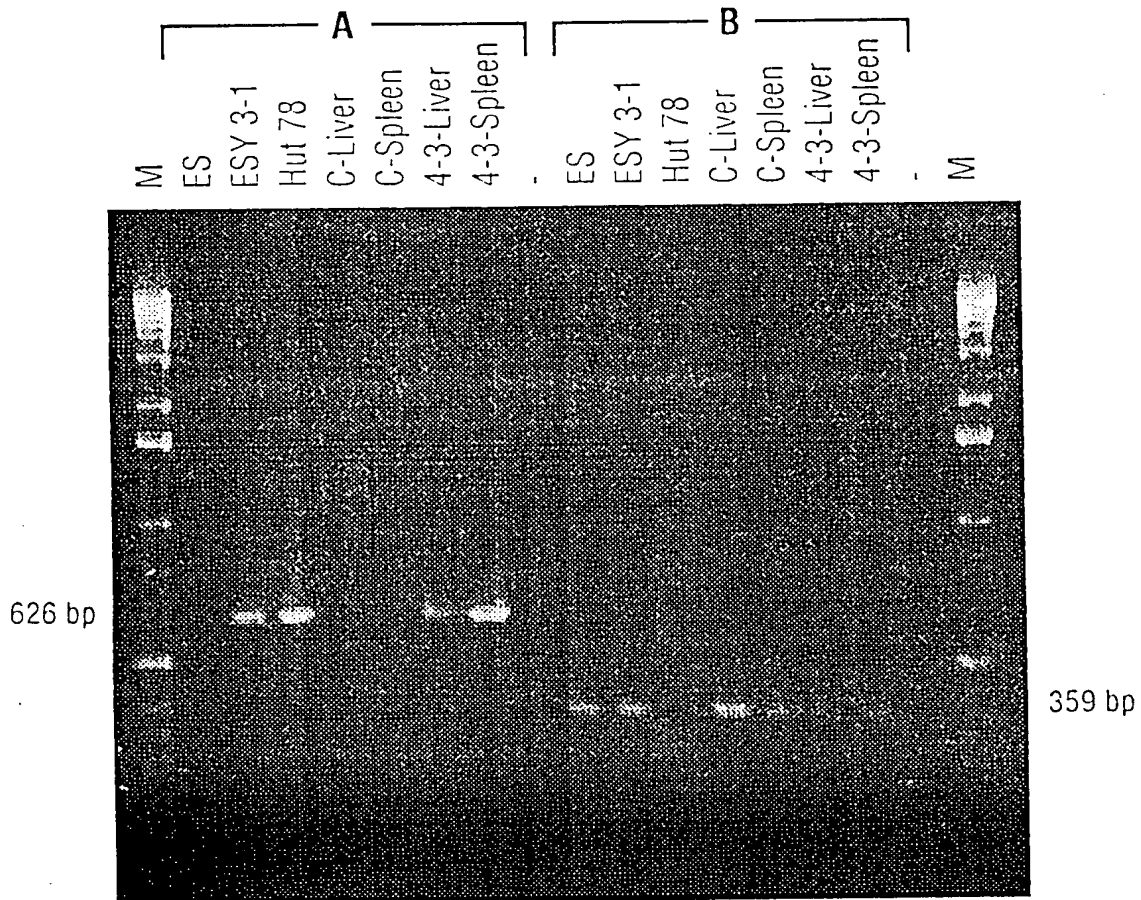
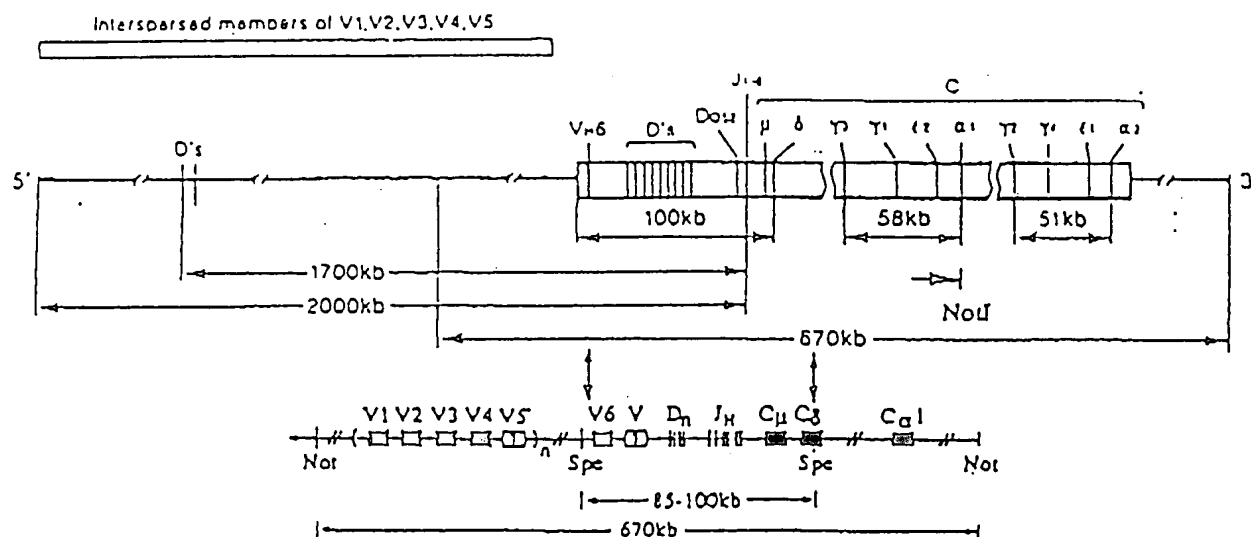


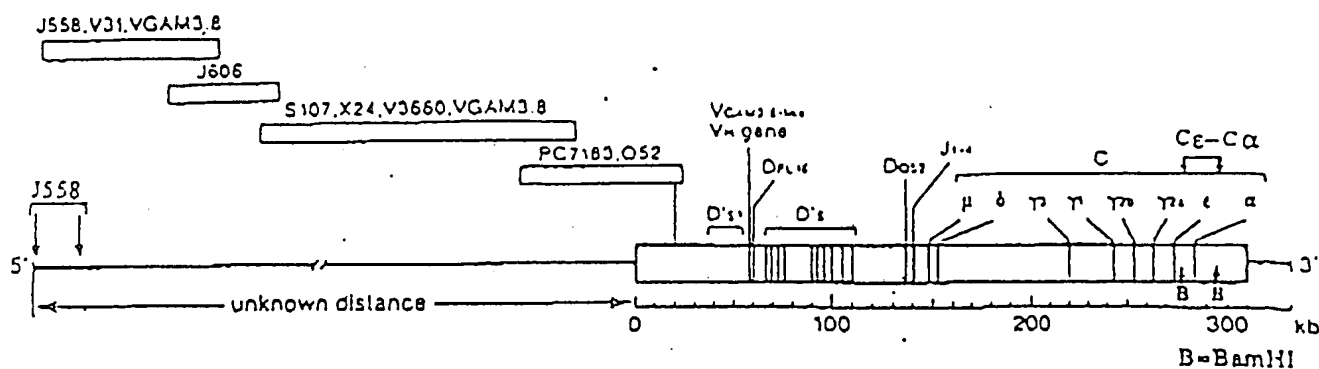
Figure 15

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(A) Human heavy chain locus



(B) Mouse heavy chain locus



(C) Human heavy chain replacement YAC vector

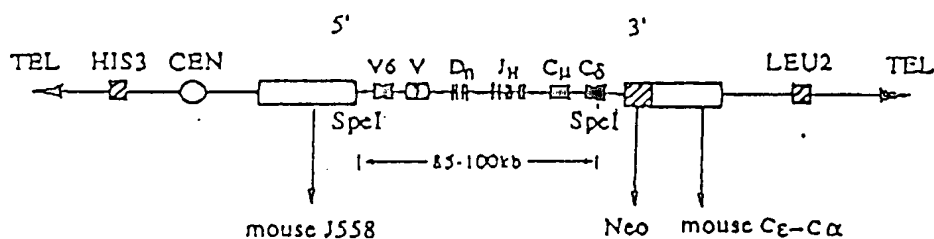


Figure 16

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Mouse Breeding Scheme

Cross IA.

heterozygous inactive Murine IgH
X
heterozygous inactive Murine IgK

MIgH (inactive) MIgK
MIgH MIgK

X

MIgH MIgK (inactive)
MIgH MIgK

↓

F1 (cross I A)

MIgH (inactive) MIgK (inactive)
MIgH MIgK

Cross I B.

heterozygous Human IgH
X
heterozygous Human IgK

MIgH MIgK HIgH
MIgH MIgK

X

MIgH MIgK HIgK
MIgH MIgK

↓

F1 (cross I B)

MIgH MIgK HIgH HIgK
MIgH MIgK

Cross II.

F1 (cross I A) x F1 (cross I B)

↓

F2 Quadruple Heterozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH MIgK

Cross III.

Intercross F2 mice

↓

F3 DOUBLE Homozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH (inactive) MIgK (inactive)

Figure 17

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MAMMALIAN HOST GENOTYPES

<u>Hetero- or Hemi-zygous Mice</u>	<u>Intercross Product Mice*</u>
I. $\frac{\Delta mIgL}{mIgL} \frac{mIgH}{mIgH}$	$\frac{\Delta mIgL}{\Delta mIgL} \frac{mIgH}{mIgH}$
II. $\frac{mIgL}{mIgL} \frac{\Delta mIgH}{mIgH}$	$\frac{mIgL}{mIgL} \frac{\Delta mIgH}{\Delta mIgH}$
III. $\frac{mIgL}{mIgL} \frac{mIgH}{mIgH} \frac{hIgH}{hIgH}$	$\frac{mIgL}{mIgL} \frac{mIgH}{mIgH} \frac{hIgH}{hIgH}$
IV. $\frac{mIgL}{mIgL} \frac{mIgH}{mIgH} \frac{hIgL}{hIgL}$	$\frac{mIgL}{mIgL} \frac{mIgH}{mIgH} \frac{hIgL}{hIgL}$
V. Animal I X Animal II	
$\frac{\Delta mIgL}{mIgL} \frac{mIgH}{\Delta mIgH}$	$\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH}$
VI. Animal III X Animal V	
$\frac{mIgL}{\Delta mIgL} \frac{mIgH}{\Delta mIgH} \frac{hIgH}{hIgH}$	$\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgH}{hIgH}$ and $\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgH}{hIgH}$
VII. Animal IV X Animal V	
$\frac{mIgL}{\Delta mIgL} \frac{mIgH}{\Delta mIgH} \frac{hIgL}{hIgL}$	$\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL}$ and $\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL}$
VIII. Animal VI X Animal VII	
$\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$	$\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$
$\frac{mIgL}{\Delta mIgL} \frac{mIgH}{\Delta mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$	$\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$ and $\frac{\Delta mIgL}{\Delta mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$
IX. Animal III X Animal IV	
$\frac{mIgL}{mIgL} \frac{mIgH}{mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$	$\frac{mIgL}{mIgL} \frac{mIgH}{mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$
X. Animal II X Animal IX	
$\frac{mIgL}{mIgL} \frac{\Delta mIgH}{mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$	$\frac{mIgL}{mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$ and $\frac{mIgL}{mIgL} \frac{\Delta mIgH}{\Delta mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$
XI. Animal I X Animal IX	
$\frac{\Delta mIgL}{mIgL} \frac{mIgH}{mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$	$\frac{\Delta mIgL}{\Delta mIgL} \frac{mIgH}{mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$ and $\frac{\Delta mIgL}{\Delta mIgL} \frac{mIgH}{mIgH} \frac{hIgL}{hIgL} \frac{hIgH}{hIgH}$

*Not all possible genotypes from intercrosses are shown.

Δ = functionally inactive locus
m = mouse endogenous gene
h = human transgene
IgH = immunoglobulin heavy chain
IgL = immunoglobulin light chain

FIGURE 18